

Effect of Steam Heating Alfalfa Hay on Utilization by Lactating Dairy Cows¹

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ABSTRACT

Alfalfa hay was steam heated for an average of 47 min at 100 to 110°C; net ruminal protein escape (total escape minus ADIN \times 6.25), estimated in vitro, was 50% versus 29% for control hay. Heating increased ADIN content from 4.6 to 15.3% of total N and increased NDF from 43 to 53% of DM. In trial 1, 14 cows averaging 81 DIM and 30 kg/d of milk were fed, in a switchback design, diets containing 18% corn, .7% urea, and 81% control or heated hay. Intake of DM, milk fat content, and fat yield were unaffected by hay source. However, feeding heated hay reduced production of milk; yields of protein, lactose, and SNF; digestibility of DM and N; and ruminal concentrations of ammonia and total VFA. In trial 2, 16 cows averaging 36 DIM and 38 kg/d of milk were fed, in balanced 4 \times 4 Latin squares, diets containing 1) 76% alfalfa silage and 23% high moisture corn; 2) 76% alfalfa silage, 14% high moisture corn, and 9% soybean meal; 3) 52% alfalfa silage, 23% high moisture corn, and 24% control hay; and 4) 43% alfalfa silage, 32% high moisture corn, and 24% heated hay. High moisture corn content of the diet with heated hay was increased to equal-

ize NE_L. Reduced ruminal ammonia and branched-chain VFA indicated decreased ruminal protein degradation on heated hay. Intake of DM, production of milk, and yields of protein, lactose, and SNF were greatest on heated hay, intermediate on soybean meal, and lowest on alfalfa silage and unheated hay. Digestibility of DM and ruminal pH were slightly lower on heated hay. Results indicated that steam heating alfalfa hay improved protein utilization but reduced energy digestibility.

(Key words: alfalfa hay, protein utilization, heat treatment, milk production)

Abbreviation key: AP = absorbable protein, C = control diet, H = heat-treated alfalfa hay, HMC = high moisture corn, SBM = soybean meal, SRF = strained ruminal fluid, U = unheated alfalfa hay, UIP = undegraded intake protein.

INTRODUCTION

Alfalfa forage is useful for feeding dairy cattle because of its high protein content. However, alfalfa protein often is utilized poorly by ruminants because of extensive degradation in the rumen (2). Heat treatment of oil seeds enhanced protein resistance to ruminal degradation and improved cow performance (8, 12). Heating of alfalfa also may improve protein utilization. Replacement of soybean meal (SBM) protein with protein from alfalfa that had been heat processed by dehydration reduced ruminal branched-chain VFA, indicating reduced ruminal degradation, and increased plasma branched-chain AA, indicating increased intestinal AA absorption (21). Partial replacement of high moisture alfalfa silage in the diet with dehydrated alfalfa increased milk production (23). Goering and Lindahl (14)

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reported that lambs fed alfalfa hay that had been dehydrated at 120 or 145°C had improved digestibility and growth compared with those fed barn-dried or 180°C dehydrated alfalfa hays. However, overheated alfalfa increased ADIN and NDF contents (30, 31) and reduced apparent digestibilities of DM, N, N-free extract, and ADF (16, 32).

Whether heat treatment of alfalfa hay improves protein utilization in lactating cows remains uncertain. We have in vitro evidence that both dry heating and steam heating of alfalfa hay reduced ruminal protein degradation (29). The objective of this study was to determine whether the previously devised steam treatment of alfalfa hay would improve performance when it was fed to lactating cows. Steam-treated alfalfa hay was fed in two trials as part of diets that were limiting in protein (2): 1) with hay fed as all of the forage or 2) with hay fed as a portion of the forage when the balance of the dietary forage came from alfalfa silage.

MATERIALS AND METHODS

Heat Treatment of Alfalfa Hay

Second-cutting alfalfa hay was cut and conditioned (model 1495; Ford-New Holland, New Holland, PA), field-dried, and harvested as small rectangular bales at late bud to early bloom stages. A portion was set aside as unheated alfalfa hay control (U), and a portion was heat treated (H) in a large soil autoclave (Reid Boiler Works, Bellingham, WA). Previous work with steam treatment of alfalfa hay (29) indicated that the optimal process was to heat the hay for 30 to 120 min at 100°C or for 15 to 30 min at 110 to 120°C. Temperature in the autoclave was held to a maximum of 110°C by controlling steam pressure at 42.1 kPa with a dead-weight pressure release valve. The autoclave was shaped cylindrically and had a volume of 3.7 m³; the chamber was configured with wooden racks to hold 12 rectangular 23-kg bales at a time. Bales were loaded into the chamber so that their edges did not touch each other; thus, steam could penetrate each bale. Thermocouples were centered at two locations each in three representative bales, and the autoclave chamber was sealed to begin the heating cycle. Thermocouple temper-

atures were monitored in about one-half of the 24 total batches of H. About 45 min were required to reach an average of 100°C on all six thermocouples; mean heating time at 100 to 110°C was 47 min (mean total time in the autoclave = 92 min). After completing the heating cycle, cooling was begun by opening the chamber door; hay was removed from the autoclave within 5 min. The DM content of H had equilibrated to that of U within 2 h after removal from the autoclave. Weekly composite samples were prepared by coring about 15 bales of each hay being fed during each week of trials 1 and 2; 8 samples were collected from U and 4 from H during trial 1 and 4 each from U and H during trial 2. Composites were ground through a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA), and duplicate samples were analyzed for DM, CP, and ash (1); NDF and ADF (22); and for proportion of total N present as ADIN (15). Duplicate samples (.250 g) were extracted with 25 ml of distilled water with swirling for 60 min at room temperature; extracts were filtered through Whatman number 1 filter paper (Whatman, Clifton, NJ), and filtrates were analyzed for total reducing sugars using glucose as standard (28). The NE_L contents of the hays were computed from their NDF (18). Each composite also was assayed for fractional rate of protein degradation, degradable CP (fraction B), and proportion of protein escaping the rumen using an inhibitor in vitro system, including a correction for unavailable CP (fraction C); fraction C was assumed to be equivalent to ADIN × 6.25 (4, 29). Net extents of ruminal protein escape (i.e., corrected for unavailable CP) were computed using the equation

$$\text{estimated protein escape (\%)} = B \times [k_p / (k_d + k_p)]$$

where k_p , the ruminal passage rate, was assumed to be equal to .06/h. Significance of composition differences because of heat treatment and trial was tested using the general linear models of SAS (24). Means for these assays are in Table 1.

Trial 1

Fourteen multiparous Holstein cows at (means ± SE) 600 ± 17 kg of BW, parity 2.9 ±

TABLE 1. Mean composition of unheated and heat-treated alfalfa hay fed during trial 1 (n = 8) and trial 2 (n = 4).

Component	Trial 1		Trial 2		P > F ¹
	Unheated	Heated	Unheated	Heated	
DM, %	91.3	92.3	91.9	93.0	.123
Reducing sugars, mmol/100 g of DM	29.1	8.7	28.2	10.2	<.001
CP, % of DM	18.4	18.4	17.9	18.9	.452
NDF, % of DM	42.8	54.3	42.7	51.0	<.001
ADF, % of DM	30.8	35.1	31.6	32.5	.017
ADIN, % of TN ²	4.4	16.3	4.8	14.3	<.001
NE _L ³ Mcal/kg of DM	1.40	1.15	1.40	1.22	<.001
Degradation rate (k _d) ⁴ /h	.136	.034	.117	.037	<.001
Estimated net escape ⁵ %	28	50	30	49	<.001

¹Probability of a significant heat treatment effect.

²TN = Total N.

³Values of NE_L computed from NDF using the equation of Mertens (18).

⁴Ruminal degradation rate determined with an inhibitor in vitro system (4).

⁵Estimated net ruminal escape (%) = $[B \times (k_p / (k_p + k_d))]$, where B = net degradable protein (i.e., corrected for ADIN $\times 6.25$) and the ruminal passage rate, k_p , is assumed to be = .06/h (4).

.3, 81 ± 6 DIM, and $29.5 \pm .6$ kg/d of milk were blocked into two groups of nearly equal average production and stage of lactation and used in a switchback lactation study. Two cows equipped with permanent ruminal cannulas were assigned to each group, 1 each from the 14 lactation study cows plus 1 each at relatively low production (about 15 kg/d of milk). The trial was divided into four 2-wk periods. During period 1, all cows were fed only U as forage. Periods 2 and 3 were the switchback (2×2 Latin square) part of the trial; during period 2, group 1 was fed U, and group 2 was fed H; hays were switched during period 3. During period 4, all cows again were fed only U as forage. The study was designed to test for responses to protein supply. Milk production responses to postruminal protein infusion are very rapid, occurring within 24 h (6). Hence, 1 wk was considered to be adequate for adaptation to protein supplementation; production and intake data from the last week of each period were analyzed statistically. Milk production was recorded daily at both a.m. and p.m. milkings. Milk samples were collected at one a.m. and p.m. milking midway through wk 2 of each period and analyzed for percentages of fat, protein, lactose, and SNF by infrared analysis (Wisconsin DHI Cooperative, Madison) and for urea (3). Cows were weighed on 3 consecutive d at the start of the trial and at the end of each period.

Diets contained (DM basis) about 81% from either U or H, 18% cracked corn, plus .7% urea (Table 1); hays were chopped to a theoretical length of 6.0 cm, blended with other ingredients, and fed as TMR. Feed was offered twice daily at 0800 and 1600 h, and orts were recorded once daily. Feed offered was adjusted daily to yield 5% orts. Weekly composites of corn and of each type of hay, TMR, and orts were collected from daily samples of about .5 kg and stored at 22°C.

Four hours after feeding on d 13 of each period, blood samples were taken from the 14 lactation study cows from the coccygeal artery or vein. Blood was heparinized and stored at 2°C for about 4 h when plasma was prepared and deproteinized using 4:1 plasma:15% (wt/vol) 5-sulfosalicylic acid and then stored at -20°C. Deproteinized plasma was analyzed for glucose and urea (3). Also on d 13, a single fecal grab sample was collected from each of the 16 cows, and ruminal samples were taken from the 4 ruminally cannulated cows. Strained ruminal fluid (SRF), taken from the ventral sac at 0 (just prior to feeding), 1, 2, 3, 4, and 6 h after feeding, was prepared by straining ruminal contents through two layers of cheesecloth. The pH was determined immediately; SRF then was preserved by addition of 1 ml of 50% (vol/vol) sulfuric acid per 50 ml of SRF. Feces and acidified SRF were stored at -20°C. Samples of SRF were thawed

and centrifuged at $30,000 \times g$ for 15 min at 2°C ; supernatants were analyzed for ammonia and total AA (5) and for individual and total VFA by gas chromatography using α -ethyl-n-butyrate as internal standard (3).

Composite samples of diet ingredients and TMR were ground through a 1-mm Wiley mill screen and analyzed for CP by Kjeldahl using a copper digestion catalyst (Kjeltabs; Tecator Inc., Herndon, VA), DM at 105°C , and ash (1), NDF and ADF (22), indigestible ADF (10), and ADIN (15). Samples of orts were analyzed for DM at 105°C (1), and DMI is reported on this basis. Feces were thawed and dried at 60°C for 72 h; ground through a 1-mm Wiley mill screen; and analyzed for DM, NDF, ADF, total N, ADIN, and indigestible ADF. Total tract apparent digestibilities were calculated using indigestible ADF as internal marker (9). Compositions of rations computed from analyses of feed ingredients are in Table 2.

Differences between groups for each experimental variable were tested during periods 1 and 4 using a *t* test (26). Significant difference ($P = .10$; $t = 1.78$ for $df = 12$ for production data from the 14 cows, $t = 1.76$ for $df = 14$ for digestibility estimates from all 16 cows, and $t = 2.92$ for $df = 2$ for ruminal measurements from the 4 cannulated cows) during period 1 was used as the criterion to test whether groups were unequally matched. If the *t* test was not significant for both periods 1 and 4, this was taken as evidence that groups were equally matched and that there was no carry-over from the switchback periods 2 and 3 for that experimental variable. This test showed group differences only for milk urea. However, *t* values were significant for period 1 ($t = 2.23$) and period 4 (2.32), indicating that the inherently higher milk urea concentrations in group 1 were not influenced by carry-over from periods 2 and 3. Differences attributed to dietary U or H during periods 2 and 3 were tested with a 2×2 Latin square design, replicated seven or two times (for ruminal data) using the general linear models of SAS (24) and including group, cow within group, period, and treatment (hay source) in the model.

Trial 2

Sixteen Holstein cows with 591 ± 10 kg of BW, parity $3.5 \pm .4$, 36 ± 2 DIM, and $38.0 \pm$

2.1 kg/d of milk were blocked into four groups of 4 cows each with nearly equal stage of lactation, production, and parity; cows were assigned randomly to four balanced 4×4 Latin squares. Four additional cows at low production (≤ 20 kg/d of milk), fitted with permanent ruminal cannulas, were used for ruminal sampling in a single 4×4 Latin square. Diets were fed as TMR and contained (DM basis) 76% alfalfa forage from either wilted third-cutting alfalfa silage or silage plus U or 67% alfalfa forage from silage plus H. Concentrate was mainly high moisture corn (HMC). Diets were fed for 2-wk periods before switching (total 8 wk); the 1st wk was considered to be transitional (6), and production and intake data analyzed were from the 2nd wk of each period. The four diets fed in the Latin squares were the following (Table 2): 1) control (C), 76% alfalfa silage plus 23% HMC (no protein supplement); 2) 76% alfalfa silage, 14% HMC, plus 9% SBM; 3) 52% alfalfa silage, 23% HMC, plus 24% U; and 4) 43% alfalfa silage, 32% HMC, plus 24% H. The amount of CP from SBM in diet 2 equaled that in U and H in diets 3 and 4. The percentage of HMC in diet 4 was increased so that its NE_L content was about equal to the other diets, based on computed NE_L of U and H (18) and tabulated NE_L (19) of other ingredients. Silage and HMC contents of as-fed rations were adjusted at the beginning of each period based on DM determined at 55°C (48 h). Weekly composites of alfalfa silage, HMC, each TMR, and type of orts were collected from daily samples of about .5 kg and stored at -20°C . Actual proportions of DM from each component were computed from DM determined by toluene distillation for silage and HMC (11) and at 105°C (1) for U and H.

Alfalfa silage was wilted to 51% DM, chopped to a theoretical length of 1.0 cm, and stored in a bunker silo. Alfalfa silage contained (DM basis) 23% CP, 12% ash, 39% NDF, 29% ADF, 1.48 Mcal NE_L /kg of DM (18), and (total N basis) 48% NPN and 6.0% ADIN. For computation of TMR undegraded intake protein (UIP), alfalfa silage UIP was assumed to be equal to 20% of CP (25), rather than 23% of CP (19), and HMC UIP was assumed to be equal to 42% of CP, the mean of NRC values [Table 7-3 in (19)] for corn grain and corn silage. Preparation of TMR, determination of

TABLE 2. Composition of diets.¹

Item	Trial 1		Trial 2			
	U	H	C	SBM	U	H
			(% of DM)			
Alfalfa silage	75.9	75.9	51.8	42.5
Cracked corn	17.5	17.5
Urea	.7	.7
High moisture corn	22.8	13.9	23.3	32.2
Soybean meal	8.9
U	80.5	23.6	...
H	...	80.6	24.0
Dicalcium phosphate	.7	.7	.7	.7	.7	.7
TMS + Se ²	.5	.5	.5	.5	.5	.5
Vitamin premix ³	.1	.1	.1	.1	.1	.1
Chemical composition						
CP	18.5	18.5	19.5	22.9	18.2	17.1
NDF	36.0	45.3	33.7	33.7	34.5	34.5
ADF	25.4	28.8	23.2	23.6	23.7	21.8
NE _L , ⁴ Mcal/kg	1.45	1.25	1.56	1.56	1.54	1.53
UIP, ⁵ % of CP	22.2	40.4	22.2	23.5	24.0	31.5

¹U = Unheated alfalfa hay; H = steam heat-treated alfalfa hay; C = control diet; SBM = soybean meal; TMS = trace-mineralized salt; UIP = undegraded intake protein.

²Provided (mg/kg of DM): Mn, 27; Zn, 27; Fe, 17; Cu, 7; I, .40; Se, .30; and Co, .10.

³Provided (IU/kg of DM): vitamin A, 3880; vitamin D, 730; and vitamin E, .73.

⁴Computed from NE_L values of U and H, alfalfa silage [1.48 Mcal/kg of DM; (18)], and NRC (19) tables.

⁵Computed using estimated net escapes for UIP for hays U and H, UIP values for cracked corn and SBM from NRC [Table 7-3; (19)], and UIP = 20 and 42% of CP for alfalfa silage and high moisture corn, respectively.

BW, DMI, milk production and composition, feed sampling and analyses, SRF sampling and analyses, fecal grab sampling and analyses, and blood sampling and analyses were as described in trial 1.

Data were analyzed as a 4 × 4 Latin square, replicated four times for DMI, BW change, milk production, and blood plasma data and replicated once for ruminal data, using the general linear models of SAS (24). The model included square, cow within square, and period within square, treatment, and period by treatment interaction. Period by treatment interactions were not significant ($P \geq .16$) for any variable tested, so they were pooled with the residual. When dietary treatment effects were significant ($P < .05$), mean separation was by least significant difference.

RESULTS

Trial 1

The composition of hays U and H fed during trial 1 are in Table 1. Steam treatment of H

increased ADF, NDF, and ADIN and decreased reducing sugars and estimated NE_L (Table 1). These changes were reflected in reduced apparent digestibility of DM and total N with feeding of H (Table 3). Although apparent digestibility of ADF was unaltered, apparent digestibility of both NDF and ADIN were substantially increased (Table 3). Steam heating decreased ruminal in vitro protein degradation rate and increased estimated net protein escape (i.e., total protein escape minus ADIN × 6.25; Table 1) by 22 percentage units. Intake of DM, BW gain, milk fat and lactose concentrations, and FCM and fat yields were unaltered ($P \geq .17$) by hay source (Table 4). However, production of milk; yields of protein, lactose, and SNF; and milk concentration of protein and SNF were all reduced significantly when cows were fed H (Table 4).

Although plasma glucose was not influenced by hay source, urea in both milk and plasma was lower with H diet (Table 4). Ruminal concentrations of ammonia, valerate, and the branched-chain VFA, isobutyrate and 2-methylbutyrate plus isovalerate (Table 5),

TABLE 3. Effect of feeding heat-treated hay on apparent digestibility¹ of dietary nutrients (trials 1 and 2).²

Item	Trial 1			Trial 2				
	U	H	<i>P</i> > <i>F</i> ³	C	SBM	U	H	<i>P</i> > <i>F</i>
	(%)							
DM	61.2	53.8	<.001	62.2 ^a	62.7 ^a	62.6 ^a	60.6 ^b	.026
OM	ND	ND	...	63.8 ^a	64.6 ^a	64.2 ^a	62.3 ^b	.014
NDF	44.2	51.6	<.001	53.0 ^a	53.1 ^a	50.5 ^b	51.4 ^{ab}	.014
ADF	40.5	41.2	.584	44.5 ^{ab}	44.9 ^a	43.0 ^b	39.5 ^c	<.001
ADIN	-12.2	35.8	<.001	-27.0 ^c	-22.2 ^{bc}	-18.0 ^b	1.8 ^a	<.001
Total N	69.0	46.7	<.001	61.4 ^b	67.2 ^a	62.1 ^b	52.4 ^c	<.001

^{a,b,c}Means with different superscripts in trial 2 differ (*P* < .05).

¹Apparent digestibility estimated using indigestible ADF as an internal marker (9).

²U = Unheated alfalfa hay; H = heat-treated alfalfa hay; C = control diet; SBM = soybean meal; ND = not determined.

³Probability of a significant treatment effect.

also were reduced with feeding of H (Table 5). Reduced urea, ruminal ammonia, and branched-chain VFA were related to decreased ruminal degradability of protein in H (Table 1). Ruminal butyrate was lower, but no differences occurred in ruminal pH, acetate, propionate, or total VFA concentrations (Table 5).

Trial 2

Differences in composition and protein degradability of U and H were essentially the

same as in trial 1, except H fed in trial 2 was slightly lower (*P* < .05) in ADIN (Table 1). Despite addition of more HMC, replacement of one-third of dietary alfalfa silage with H reduced apparent DM and OM digestibility about 2 percentage units (Table 3). Apparent digestibilities of NDF and ADF generally were lower in diets containing U and H. Apparent digestibilities of N were confounded by differences in ration CP, but the difference of about 10 percentage units between diets U and H,

TABLE 4. Effect of feeding heat-treated hay on DMI, BW gain, production of milk, yield of milk components, and concentrations of milk urea and plasma urea and glucose (trials 1 and 2).¹

Item	Trial 1			Trial 2				
	U	H	<i>P</i> > <i>F</i> ²	C	SBM	U	H	<i>P</i> > <i>F</i> ²
DMI, kg/d	22.9	23.4	.318	23.4 ^b	24.2 ^{ab}	23.5 ^b	25.2 ^a	.003
BW Gain, kg/d	.22	.02	.506	.27	-.03	-.08	.69	.281
Milk, kg/d	28.2	27.2	.002	33.4 ^c	35.1 ^{ab}	34.1 ^{bc}	35.7 ^a	.018
3.5% FCM, kg/d	26.7	26.5	.736	34.7	35.3	36.6	35.7	.469
Fat, %	3.17	3.34	.168	3.69	3.59	3.83	3.52	.284
Fat, kg/d	.90	.91	.707	1.22	1.26	1.31	1.25	.574
Protein, %	3.07	3.01	<.001	2.96	2.95	2.92	2.97	.626
Protein, kg/d	.87	.82	.001	.98 ^b	1.03 ^{ab}	.99 ^b	1.06 ^a	.053
Lactose, %	4.68	4.65	.234	4.84	4.85	4.85	4.87	.560
Lactose, kg/d	1.32	1.26	.001	1.61 ^c	1.70 ^{ab}	1.65 ^{bc}	1.74 ^a	.010
SNF, %	8.43	8.34	.013	8.44	8.44	8.41	8.49	.543
SNF, kg/d	2.38	2.27	<.001	2.81 ^c	2.96 ^{ab}	2.86 ^{bc}	3.02 ^a	.019
Milk urea, mM	6.35	4.87	<.001	6.95 ^b	8.46 ^a	6.25 ^c	5.30 ^d	<.001
Plasma urea, mM	7.97	5.65	<.001	7.12 ^b	9.81 ^a	7.02 ^b	5.13 ^c	<.001
Plasma glucose, mg/dl	68.7	71.9	.256	62.5	62.6	61.8	62.6	.905

^{a,b,c,d}Means with different superscripts in trial 2 differ (*P* < .05).

¹U = Unheated alfalfa hay; H = heat-treated alfalfa hay; C = control diet; SBM = soybean meal.

²Probability of a significant dietary treatment effect.

TABLE 5. Effect of feeding heat-treated hay on ruminal pH, concentrations of N metabolites, and VFA (trials 1 and 2).¹

Item	Trial 1			Trial 2				
	U	H	<i>P</i> > <i>F</i> ²	C	SBM	U	H	<i>P</i> > <i>F</i> ²
pH	6.42	6.51	.605	6.43 ^a	6.58 ^a	6.42 ^a	6.10 ^b	.011
Ammonia, mM	18.0	9.2	.007	23.4 ^{ab}	28.0 ^a	22.6 ^b	13.3 ^c	.002
Total AA, mM	.60	.59	.785	1.88	1.76	1.79	1.34	.639
Total VFA, mM	136	125	.355	121	119	113	127	.119
VFA, mol/100 mol								
Acetate (A)	68.6	69.4	.345	64.0	64.3	64.2	64.0	.537
Propionate (P)	18.7	19.8	.513	18.9	18.5	18.3	19.4	.201
Butyrate	9.6	8.3	.014	11.3	11.1	11.5	10.8	.357
Isobutyrate	1.07	.50	.014	1.59 ^b	2.07 ^a	1.40 ^b	1.00 ^c	.032
Valerate	1.62	1.22	.006	1.96	2.01	1.93	1.80	.190
2-Methylbutyrate								
+ isovalerate	1.39	.45	.008	2.09 ^{ab}	2.39 ^a	1.70 ^{bc}	1.50 ^c	.019
A:P	3.68	3.54	.662	3.43	3.51	3.54	3.38	.166

^{a,b,c}Means with different superscripts in trial 2 differ (*P* < .05).

¹U = Unheated alfalfa hay; H = heat-treated alfalfa hay; C = control diet; SBM = soybean meal.

²Probability of a significant dietary treatment effect.

which differed only by 1% CP (Table 2), indicated that N digestibility was depressed in cows fed diet H (Table 3). Apparent digestibility of ADIN was lowest on diet C, intermediate on diets SBM and U, and greatest on diet H (Table 3).

Feed DMI, BW change, milk production, milk and plasma urea, and plasma glucose data are in Table 4. Intake averaged about 1.7 kg/d of DM more on diet H than on diets C and U; DMI was intermediate on the SBM diet. Change in BW was not influenced by diet. Increased intake on diet H was reflected in greater production of milk and yields of lactose, SNF (*P* ≤ .02), and protein (*P* = .053) than on diets C and U; production was intermediate on the SBM diet. Diet had no effect on yields of FCM or fat or on concentration of any milk component. Milk and plasma urea (Table 4) followed the pattern of total N digestibility (Table 3); highest on diet SBM, intermediate on diets C and U, and lowest on diet H. Blood glucose was unaffected by diet.

Ruminal pH and ammonia concentration were lowest on diet H; ruminal ammonia was intermediate on diets C and U (Table 5). As with ammonia, ruminal concentrations of the branched-chain VFA, isobutyrate and 2-methylbutyrate plus isovalerate, were lowest with diet H, intermediate on diets C and U, and highest on the SBM diet (Table 5). Rumi-

nal concentrations of ammonia and branched-chain VFA followed milk and plasma urea concentrations and apparent N digestibility and were in proportion to dietary CP percentages (Table 2). No other VFA was affected by diet.

DISCUSSION

The estimated UIP of 29% for U (Table 1), measured by an *in vitro* procedure (4), was similar to that reported by the NRC (19). Even after discounting for 15% ADIN, estimated UIP of the steam-treated H averaged 21 percentage units more than that of U. Reduced ruminal ammonia and branched-chain VFA (Table 5) indicated clearly that the steam treatment reduced *in vivo* degradation of protein in H (3). However, steam treatment also reduced the energy value of H: NDF was increased nearly 13 percentage units, and estimated NE_L (18) was reduced 16% (Table 1). The NE_L, which was computed from NDF content, may be underestimated because apparent NDF digestibility was increased with H in trial 1 (Table 3). The decreased *in vivo* digestibilities of DM and N in trial 1, although expected (32), were greater than anticipated. The ADIN and NDF concentrations observed for H were similar to those reported earlier by Yu (30, 31) and Goering and Lindahl (14) for alfalfa hay judged to be heat damaged. Compared with U fed at 81% of the diet, the H diet clearly

depressed milk production (Table 4). That yields of FCM and fat were not lowered reflects the ability of the cow to mobilize body fat in early lactation (27).

Absorbable protein (AP) supply, computed from UIP intake (Table 2) and microbial protein synthesis estimated from NE_L intake (19), was 2.2 and 2.6 kg/d on diets U and H, respectively. The milk production predicted from AP supply and NE_L intake (19) during trial 1 was 27 and 32 kg/d (diet U) and 34 and 28 kg/d (diet H). Actual milk production (Table 4) was within 1 kg/d of the lower predicted amount in both cases, which suggested that the AP limitation of production on diet U was corrected by steam-treated H in diet; however, depressed energy content of H limited milk production on that diet.

Previously, we (29) found that the steam treatments that gave optimal improvement in estimated net ruminal escape of protein in control or shredded alfalfa hay (30 to 120 min at 100°C or 15 to 30 min at 110 to 120°C) also increased ADIN content to 7 to 15% of total N. Steam heating for up to 120 min at 110°C, which increased ADIN to about 17% of total N, reduced the estimated net protein escape by less than .10 of maximum because total protein escape increased by amounts comparable with the increased ADIN (29). However, at temperatures greater than 110°C, ADIN formation was excessive, reaching 32 and 39% of total N after 120 min at 120 and 130°C, respectively (29). Hay H (which had not been shredded) fed in these trials had an average ADIN content of 15.3% of total N (Table 1). This ADIN content was considered to be acceptable in that it was within the range in which additional ADIN formation was compensated for by increased total protein escape. The finding that significantly more ADIN was digested in trial 1 with diet H (Table 3) was similar to observations of Goering and Lindahl (14) and suggested that ADIN may not be a completely reliable index of unavailable N. The trend was similar in trial 2 for diet H, which had about one-third as much steam-heated hay.

Alfalfa hay may be more sensitive to overheating than other protein sources. Increases in ADIN content of H are much more rapid than with oilseeds and oilseed meals. Faldet et al. (13) found that oven heating of whole soybeans had little effect on ADIN content. Heat-

ing soybeans at 160°C for 120 min increased ADIN only to 4.5% of total N. Heat treatments of soybeans (12) or SBM (7), which increased estimated ruminal protein escape to about 60%, also improved milk production but had little effect on ADIN content. Available Lys content may be a more sensitive measure of extent of overheating in oilseeds (13). We concluded that the steam H was an effective source of UIP but that reduced energy availability impaired utilization of H when it was fed at 81% of the diet.

Improved UIP but depressed energy value suggested that H might be more effective when fed as a "protein supplement", so H was fed as one-third of the forage and compared with U or equal CP from SBM in trial 2. In trial 2, 9% more HMC also was fed (Table 2) to compensate for the depressed energy content of H. Although still significantly lower, DM and OM apparent digestibilities on diet H were within 2 percentage units of the other three diets (Table 3). Lower digestibility may explain the slightly greater DMI on H in trial 2. Digestible DMI were 14.6, 15.2, 14.7, and 15.3 kg/d for diets C, SBM, U, and H, respectively, and were unaffected by diet ($P = .18$). Thus, energy availability was more nearly equal among diets in trial 2 than in trial 1. Milk production and milk component yields were as great on diet H as on the SBM diet (Table 4), which contained 5.6 percentage units more CP (Table 2). Clearly, the SBM diet contained excess protein; however, production was significantly lower on diets C and U, which contained, respectively, 2.4 and 1.1 percentage units more CP than did diet H. Milk urea concentration on H of about 5 mM suggested that ruminal degradable protein and fermentable energy were more nearly in balance (20). Although part of the response to diet H may have been mediated through greater DMI, the relatively high milk production achieved at lower CP is noteworthy. Improved efficiency of dietary CP utilization reduces N losses in excrement and may have the desirable effect of reducing nitrate contamination of ground water, which is a problem that is now becoming important (17).

The AP supplies, computed from UIP intake (Table 2) and microbial protein synthesis estimated from NE_L intake (19), were 2.4, 2.7, 2.4, and 2.7 kg/d for diets C, SBM, U, and H,

respectively. Milk production predicted from AP supply and NE_L intake (19) was 34 and 37 kg/d (diet C), 40 and 41 kg/d (diet SBM), 34 and 39 kg/d (diet U), and 40 and 37 kg/d (diet H). Comparison of these estimates with actual milk production (Table 4) suggested that AP supply limited milk production on diets C and U and that the greater production on diet H was because of the increased UIP content of steam-heated alfalfa hay. Milk production on the SBM diet was about 5 kg/d less than predicted. Other environmental factors or genetics may have limited milk production on this trial to a maximum of 35 to 36 kg/d.

CONCLUSIONS

Heat treating alfalfa hay with steam for 47 min at 100 to 110°C reduced ruminal protein degradability but also reduced energy digestibility. This hay fed as the only forage decreased milk production in cows fed diets with 81% alfalfa. However, steam-treated H, fed to replace unheated hay or alfalfa silage at 24% of dietary DM, increased DMI, production of milk, and yield of milk components when its reduced energy content was compensated for by feeding more concentrate. Milk production in cows fed H, which had 17% CP, was comparable with that on the SBM diet containing 23% CP. Alfalfa hay appears to be more sensitive to overheating than oilseeds, but controlled heat treatment of hay shows promise as a means of protecting the protein in a portion of the dietary forage.

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